1.0 General Information

1.1 Protocol Title

Biospecimen Collection Protocol for CPTAC Phase III

Protocol Number: CPTAC PM-0021

1.2 Study Sponsor

National Cancer Institute (NCI)

Office of Cancer Clinical Proteomics Research (OCCPR)

1.3 Research Method

Research and Development

1.4 Principal Investigator(s) / Collection Sites

Multiple PI/Multiple Sites

2.0 Background Information

The Clinical Proteomic Tumor Analysis Consortium (CPTAC) sponsored by the NCI's Office of Cancer Clinical Proteomics Research is a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of robust, quantitative, proteomic technologies and workflows. The overarching goal of CPTAC is to improve our ability to diagnose, treat and prevent cancer. To achieve this goal in a scientifically rigorous manner, the NCI launched CPTAC to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, and provide this data with accompanying assays and protocols to the public.

The overall objective of CPTAC Phase III is to improve our understanding of cancer biology by conducting Proteogenomic analysis on selected cancer types (up to 10 cancer types, 200 cases each) where unanswered questions remain about the molecular biology of the disease. For this project, a case ideally includes collection of tumor tissue, adjacent tissue, and a source of germline DNA, such as blood. This analysis will supply a complementary layer of protein molecular biology that refines our knowledge of driver genes, enhances the understanding of tumor pathogenesis through proteomic subtyping, and illuminates the mechanism of dysregulation of cancer signaling networks and pathways via dynamic alterations in post-translational modifications.

The tissue collection and processing portions of this investigation will be carried out at multiple clinical sites with each site collecting cases from all tumor types of interest. Biospecimens will be collected at each site from consented subjects, will be coded to facilitate specimen tracking and processing, and collections will

require pre-surgical blood samples matched to surplus surgical tumor and normal tissues, where available. Tumor, pre-surgical blood and normal tissues will be used in proteogenomic experiments.

The purpose of this protocol is to establish the required procurement parameters for collection of the ten tumor types for the CPTAC study. This study will require collection of data on such pre-analytical variables as ischemic time, tissue stabilization, time and method of blood collection, and preservation in the LN2 freezing environment, as defined within a series of Standard Operating Procedures (SOPs) which will govern sample collection, processing, handling, data collection, shipment, and distribution.

3.0 Objectives

The CPTAC Phase III study will contribute to the goals of CPTAC by providing, from up to 10 cancers, high quality, well annotated biospecimens suitable for inclusion in genomic and proteomic studies. The analyses will result in a greater understanding of the proteome, the phosphoproteome and other post-translational modifications present in the tumors. Cancer pathways will be revealed, along with the variation in protein isoforms. The experiments are designed to help the program answer some or all of the following scientific questions:

Biological mechanisms

1. How are genomic aberrations detectable at protein level?
2. What is their effect on protein function?
3. Which events are drivers? Which are passengers?

Clinical applications

1. Can proteomic information enhance the molecular taxonomy of cancer?
2. Can genotypic information guide protein marker development?

4.0 Scope

The protocol applies to any samples submitted by a Leidos Biomed subcontractor to the Leidos Biomedical Research, Inc. CPTAC BCR.

5.0 Experimental Design

This CPTAC Phase III study will continue the proteogenomic experiments conducted in CPTAC Phase II and I. Tissue Source Sites (TSS) will collect cases from the list of cancers (Table 1) from consented patients who are undergoing evaluation (AML) or surgical treatment for one of these cancers.

Table 1- Cancers to be collected in CPTAC Phase III

TCGA code	CPTAC code	Cancer
SARC	SAR	Sarcomas
PAAD	PDA	Pancreatic Ductal Adenocarcinoma
HNSC	HNSCC	Head and Neck Squamous Cell Carcinoma
SKCM	CM	Cutaneous Melanoma
LUSC	LSCC	Lung Squamous Cell Carcinoma
LUAD	LUAD	Lung Adenocarcinoma
UCEC	UCEC	Uterine Corpus Endometrial Carcinoma
KIRC	CCRCC	Clear Cell Renal Cell Carcinoma
LAML	AML	Acute Myeloid Leukemia
GBM	GBM	Glioblastoma Multiforme

All TSSs will collect tumor tissue from the cancer types listed in Table 1. All samples will be processed according to standardized workflows and operating procedures at all facilities (see below). This study specifically aims for:

- Minimized specimen ischemic and processing time with the ischemic time recorded.
- Sufficient total material from each patient, divided to yield multiple samples suitable for processing for proteomic and genomic analysis.
- Consideration of heterogeneity of tumor tissue where possible.
- Determination of weights of individual samples to enable estimation of protein yield from a given aliquot.
- Flash freezing of specimens in Liquid Nitrogen vapor phase (maximum -140° C) with or without the use of OCT as subsequently described.
- Histological assessment and quality assurance by the TSS (through pathology review of adjacent tissue) of all tumor specimens submitted to the Biospecimen Core Resource (BCR).

This study will be conducted following Tissue Collection SOPs, many of which will be created and reviewed by an array of subject matter experts. TSS staff will need to follow a rigorous processing schedule and ensure all time points are recorded and specimens processed as requested, in order to maximize specimen integrity. The methods of tissue collection and processing will be structured to minimize (or reduce) the impact of tumor

heterogeneity on analysis, as feasible. Deviations from SOP will be recorded and maintained with the specimen data. TSSs will use tissue collection kits provided by the BCR for all specimen collections. Tumor biospecimens will undergo pathology review at the TSS to verify specimen diagnosis and ensure that submitted specimens meet CPTAC minimum tumor content/cellularity requirements. Submission Case Report Forms will be required to be completed. After the Submission Case Report Forms and de-identified pathology report have been posted in the Comprehensive Data Resource (CDR) database and a minimum number of eligible cases are available, permission will be granted to ship the cases in association with applicable slides or the provision of whole slide images of each tumor and adjacent normal biospecimen, to the CPTAC BCR for storage and dissemination for analysis. The BCR will create digital images of all pathology slides submitted by the TSSs. The CPTAC Pathology Resource Center (PRC) will subject the slide images to quality review. Upon image review and verification of qualification, the PRC will select experimental segments to distribute.

The TSSs will use a CPTAC-provided web-based interface to the CDR to enter all the data regarding collection, processing, and handling of the biospecimens, de-identified clinical information related to the donor, and pathological evaluation of the tumor. All data collected will be saved in the CPTAC CDR where information is quality reviewed, stored, and backed up following standard CPTAC requirements.

The BCR will store images from all slides created for this project. These images will be made available via a web portal for review and assessment by the CPTAC PRC. The PRC review will also be documented within the CDR.

6.0 Study Tissue Donors

General patient inclusion criteria

- Newly diagnosed, untreated patients undergoing primary cytoreductive surgery for a cancer meeting study diagnostic requirements, or evaluation for AML.
- Tumor from appropriate anatomic site only (other anatomic sites not acceptable, except as noted).
- Donor has not received any prior systemic treatment for any malignancy.

General patient exclusion criteria

- Prior history of other malignancies within the past 12 months except basal cell skin cancer.
- Any prior systemic chemotherapy or biological therapy for any cancer.
- Prior hormonal therapy within the last five years for any cancer.
- Prior radiation therapy for any prior malignancy that involves treatment to the region of interest.

6.1. Soft Tissue: Sarcoma –Study Protocol Inclusion/Exclusion Criteria

6.1.1. Inclusion Criteria

- Scheduled for surgical treatment of soft tissue mass assumed to be a sarcoma.

- The exclusion criteria of a donor having received prior treatment for a previous malignancy is waived for sarcoma cases. This means that patients with a history of chemotherapy or radiation are allowed into the CPTAC study, provided that the treatment occurred greater than 3 years prior to collection of the sarcoma. Treatment data on the prior tumor is required to include dates and treatment details.
- Able to provide informed consent for blood, surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Any

6.1.2. Exclusion Criteria:

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis within 3 years.

6.1.3. Criteria for Maintenance of Case in Study:

- Successful collection of required blood
- Successful collection of required tumor mass and normal tissue meeting the minimal dissection size and quantity
- Successful submission of required case data
- Pathologic verification of tumor as:
  - A primary sarcoma
  - ≥50% total cellularity
  - ≥80% tumor cellularity
  - ≤20% necrosis (by surface area of the entire slide)

6.2. Pancreas: Pancreatic Ductal Adenocarcinoma– Study Protocol Inclusion / Exclusion Criteria

6.2.1. Inclusion Criteria:

- Scheduled for surgical treatment for a pancreatic mass assumed or suspected to be pancreatic ductal adenocarcinoma
- Able to provide informed consent for blood, surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Any

6.2.2. **Exclusion Criteria:**

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis

6.2.3. **Criteria for Maintenance of Case in Study:**

- Successful collection of required blood
- Successful collection of required tumor mass and normal tissue, meeting the minimal dissection size and quantity
- Successful submission of required case data
- Pathologic verification of tumor as:
  - A primary pancreatic ductal adenocarcinoma
  - Pancreatic ductal adenocarcinoma cases do not have strict cellularity requirements, but all samples must be pre-screened by a pathologist to confirm the presence of tumor. CPTAC will perform deeper-than-normal sequencing to accommodate for low cellularity samples and tumor heterogeneity.

6.3. Head and Neck: Squamous Cell Carcinoma– Study Protocol Inclusion / Exclusion Criteria

6.3.1. **Inclusion Criteria:**

- Scheduled for surgical treatment for a head and/or neck mass assumed or suspected to be squamous cell carcinoma
- Able to provide informed consent for blood, surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Any

6.3.2. **Exclusion Criteria:**

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis

6.3.3. **Criteria for Maintenance of Case in Study:**

- Successful collection of required blood
- Successful collection of required draft tumor mass and normal tissue, meeting the minimal dissection size and quantity
- Successful submission of required case data
- Pathologic verification of tumor as:
  - A primary squamous cell carcinoma
  - ≥50% total cellularity
  - ≥80% tumor cellularity
  - ≤20% necrosis (by surface area of the entire slide)

6.4. Skin: Cutaneous Melanoma – Study Protocol Inclusion / Exclusion Criteria

6.4.1. **Inclusion Criteria**

- Scheduled for surgical treatment for a skin mass assumed or suspected to be cutaneous melanoma
- Tissue submitted for cutaneous melanoma cases may have been collected from either the primary tumor site or from a tumor metastasis.
- Cutaneous melanoma cases may have a history of interferon gamma treatment provided that the treatment was completed >90 days prior to collection of the tumor sample.
- Able to provide informed consent for blood, surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Any

6.4.2. **Exclusion Criteria:**

- Informed consent not provided
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis, with the exception of interferon gamma treatment provided that the treatment was completed more than 90 days prior to obtaining the tumor sample.

6.4.3. **Criteria for Maintenance of Case in Study:**

- Successful collection of required blood
- Successful collection of required tumor mass meeting the minimal dissection size and quantity

- Successful submission of required case data
- Pathologic verification of tumor as:
  - A primary or metastatic cutaneous melanoma
  - ≥50% total cellularity
  - ≥80% tumor cellularity
  - ≤20% necrosis (by surface area of the entire slide)

6.5. Lung: Non-small cell carcinoma – Inclusion / Exclusion Criteria

6.5.1. Inclusion Criteria:

- Scheduled for surgical treatment of a lung mass assumed to be either primary lung adenocarcinoma or squamous cell carcinoma
- Able to provide informed consent for blood, surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Any

6.5.2. Exclusion Criteria:

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis

6.5.3. Criteria for Maintenance of Case in Study:

- Successful collection of required blood
- Successful collection of required tumor mass and normal tissue meeting the minimal dissection size and quantity
- Successful submission of required case data
- Pathologic verification of tumor as:
  - A primary lung adenocarcinoma or squamous cell carcinoma
  - ≥50% total cellularity
  - ≥80% tumor cellularity
  - ≤20% necrosis (by surface area of the entire slide)

6.6. Uterine Corpus: Endometrial Carcinoma – Study Protocol Inclusion / Exclusion Criteria

6.6.1. **Inclusion Criteria:**

- Scheduled for surgical treatment for a mass assumed to be primary uterine corpus endometrial carcinoma
- Able to provide informed consent for blood, and surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Female

6.6.2. **Exclusion Criteria:**

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis
- Sex: Male

6.6.3. **Criteria for Maintenance of Case in Study:**

- Successful collection of required blood
- Successful collection of required tumor mass and normal endometrial tissue (when available) meeting the minimal dissection size and quantity
- Successful submission of required case data
- Pathologic verification of tumor as:
  - Uterine corpus endometrial carcinoma (all subtypes, any stage and grade)
  - ≥50% total cellularity
  - ≥80% tumor cellularity
  - ≤20% necrosis (by surface area of the entire slide)

6.7. Kidney: Clear Cell Renal Cell Carcinoma –Study Protocol Inclusion / Exclusion Criteria

6.7.1. **Inclusion Criteria**

- Scheduled for surgical treatment of kidney mass assumed to be a primary clear cell renal cell carcinoma
- Able to provide informed consent for blood, surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Any

6.7.2. **Exclusion Criteria:**

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis
- History of a transplanted kidney

6.7.3. **Criteria for Maintenance of Case in Study:**

- Successful collection of required blood
- Successful collection of required tumor mass and normal tissue meeting the minimal dissection size and quantity
- Successful submission of required case data
- Pathologic verification of tumor as:
  - A primary clear cell renal cell carcinoma
  - ≥50% total cellularity
  - ≥80% tumor cellularity
  - ≤20% necrosis (by surface area of the entire slide)

6.8. Hematologic Tissue: Acute Myeloid Leukemia – Inclusion / Exclusion Criteria

6.8.1. **Inclusion Criteria:**

- Diagnosed with acute myeloid leukemia
- Scheduled for bone marrow aspirate or peripheral blood draw
- Able to provide informed consent for collection of biospecimens (including bone marrow aspirate (preferred) or peripheral blood, and either skin punch biopsy or 20 micrograms DNA extracted from a buccal swab as a source of germline DNA) and associated data
- Meets age of majority for institution/state
- Sex: Any

6.8.2. **Exclusion Criteria:**

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis

6.8.3. **Criteria for Maintenance of Case in Study:**

- Successful collection of required biospecimens
- Successful submission of required case data
- Pathologic verification of diagnosis as:
  - Acute myeloid leukemia
  - Blasts comprise ≥20% of nucleated cells in the submitted specimen, either bone marrow (preferred) or peripheral blood

6.9. Brain: Glioblastoma Multiforme –Study Protocol Inclusion / Exclusion Criteria

6.9.1. **Inclusion Criteria**

- Scheduled for surgical treatment of brain mass assumed to be primary glioblastoma multiforme
- Able to provide informed consent for blood, surgical tissue donation and associated data
- Meets age of majority for institution/state
- Sex: Any

6.9.2. **Exclusion Criteria:**

- Informed consent not provided
- Tumor of experimental focus is a metastasis from another tissue or organ
- Participant already received or is undergoing chemotherapy, radiation therapy and/or immunotherapy for any previous or current cancer diagnosis

6.9.3. **Criteria for Maintenance of Case in Study:**

- Successful collection of required blood
- Successful collection of required tumor mass meeting the minimal dissection size and quantity
- Normal tissue not required
- Successful submission of required case data
- Pathologic verification of tumor as:
  - A primary glioblastoma multiforme
  - ≥50% total cellularity

- ≥80% tumor cellularity
- ≤50% necrosis (by surface area of the entire slide)

7.0 Biospecimen Collection Requirements

For each case accrued, the TSSs shall provide biospecimen sets that meet the following criteria. Minimum requirements for a complete case consist of adequate tumor tissue, normal adjacent tissue, and a source of germline DNA. All three specimen types must be available for every case, unless otherwise noted as an approved exception in Appendix B. All biospecimens will be collected according to defined SOPs (see reference documents). General guidelines for blood and tissue samples are given below.

7.1. Blood Samples

Blood samples will be collected from all study donors for the preparation of germline DNA. Blood collected for future genomic and proteomic analyses will be frozen unprocessed in designated collection tubes included in BCR-supplied kits until shipment to the BCR where specimens will be centrally stored for processing.

7.1.1. General blood sample requirements are as follows:

- Whole blood - ~10.0 mL blood draw volume

Note: Assuming a normal white blood cell count and optimal cell recovery techniques, one 10-ml tube of blood is sufficient for the recovery of 20 micrograms of DNA, the required amount for CPTAC analyses. If the white count is low or the cell recovery techniques are suboptimal, more blood may be required.

Note: For AML, tissue skin punch biopsy or 20 micrograms of DNA from a buccal swab may be submitted in lieu of blood (prior approval required if sole source of normal). See Appendix B for more information.

- The blood collected for DNA will be frozen at the TSS in Liquid Nitrogen vapor phase (maximum -140° C) and shipped to the BCR for storage and processing.

7.1.2. Blood Collection Procedure

- Obtain 10 ml of peripheral whole blood collected by standard venous phlebotomy. The blood should be collected into a labeled tube (Yellow-top tube, Becton-Dickinson CPT, sodium citrate) as provided with the biospecimen procurement kit.

- Whole blood specimens should be frozen in Liquid Nitrogen Vapor Phase within 1 hour of collection.

7.2. Surgical Tissue Collection

7.2.1. Primary Tumor Samples

- Biospecimens are to be snap frozen in Liquid Nitrogen vapor phase (maximum -140° C). Depending on the type of QC to be done, tumor specimens may be frozen with or without the use of OCT embedding media as noted below.
- To minimize total ischemic time for the tumor samples, process the tumor material prior to processing collected normal tissue.
- Sufficient tumor tissue is to be acquired to yield a minimum of 1000 ug of protein and 20 ug of co-isolated DNA and RNA. Depending on the extraction efficiency from tissue materials for their molecular contents, it is estimated that approximately 200 – 250 mg wet tissue-without OCT or other additives-will be required.
- If multiple pieces of tumor are submitted to achieve the required amount, it is not a requirement that they be contiguous, however, they must have been collected from the same tumor nodule and as close in proximity to each other as possible.
- Only tissue collected from the primary tumor may be submitted as the qualifying tumor specimen for a case, unless otherwise noted (e.g. melanoma). The collection of tissue from a metastatic site is generally not acceptable as the only source of a tumor specimen. Optionally, neoadjuvantly treated recurrent tumors and/or metastases are requested, but only when case-matched with a primary, untreated specimen (may have been previously submitted).
- The time from cutoff of in vivo blood supply (devascularization/clamp time) to ex vivo stabilization (freezing) must be no greater than 30 minutes; however, preservation within 15 minutes or less is preferred. For all cases, this total ischemic time must be documented.
- Tissue will be divided into segments measuring approximately 1.0 x 1.0 x 0.5 cm in preparation for embedding in OCT, as required below, and snap freezing.
- The overall estimate of the weight available from qualified tissue must be greater than 200 mg for submission to the BCR.

7.2.2. Normal (Non-malignant) Adjacent Tissue Samples

- Normal adjacent tissue must be provided unless an exception is listed in the table in Appendix B.
- Two to three frozen aliquots of normal tissue with a combined weight of at least 200 mg is to be submitted.

- For normal tissue, the time from cutoff of in vivo blood supply (devascularization) to ex vivo stabilization (freezing) must be no greater than 35 minutes; however, tissue preservation within 15 minutes or less is preferred. For all cases, this total ischemic time must be documented.
- Normal tissue from a concurrently excised tissue distinct from that of the primary tumor may be acceptable with pre-approval from the Leidos Biomed TPM.

7.2.3. Tissue Procurement Procedure

The excision and division of the tumor and normal tissues is to be performed as detailed within project Tissue Collection SOPs. Summary guidance is as follows:

7.2.3.1. **For TUMOR TISSUE ONLY**, when feasible, snap freeze tumor tissue and prepare corresponding adjacent tumor tissue for QC as follows:

- Obtain a 1 cm^3 piece of tumor tissue (see Diagram 1: sectioning diagram below).
- Divide the tissue to obtain two 1cm x 1cm x ~2-3 mm pieces from opposing ends of the tissue and a central piece of tissue measuring 1cm x 1cm x ~5mm.
- The two flanking pieces of tissue should be formalin fixed and processed to generate H&E stained slides (FFPE QC) for diagnostic confirmation and specimen qualification. These outer pieces should be placed in cassettes for fixation and processing, having been positioned such that the portion of tissue abutting the central piece is face down in the cassette. The central piece of tissue is to be frozen (without OCT), and submitted for analysis as the tumor specimen.
- After obtaining specimen weights for each segment to be frozen, enclose tumor tissue within a pre-chilled, labeled cryosette and immerse in the vapor phase of liquid nitrogen for 3 to 5 minutes. **Note: Do not use OCT when freezing the tissue if adjacent tissue will be fixed in formalin for QC review**.
- Store cryosettes in vapor phase liquid nitrogen until shipment.

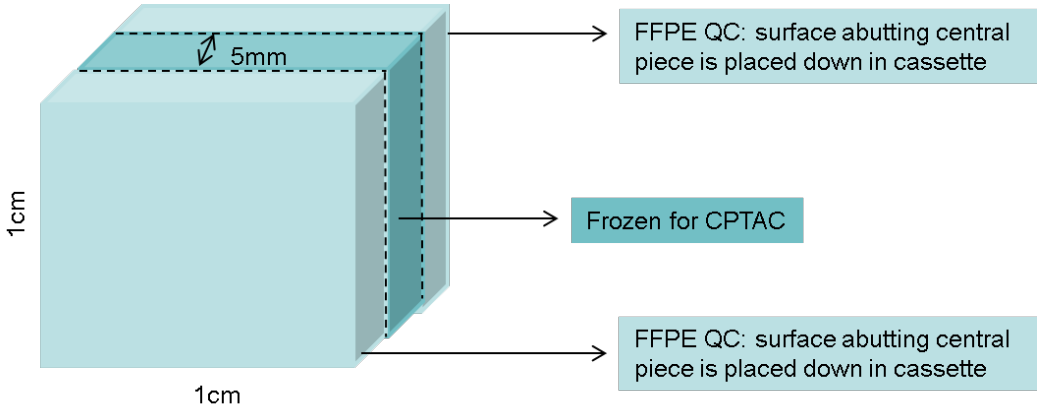


Diagram 1. Sectioning Diagram

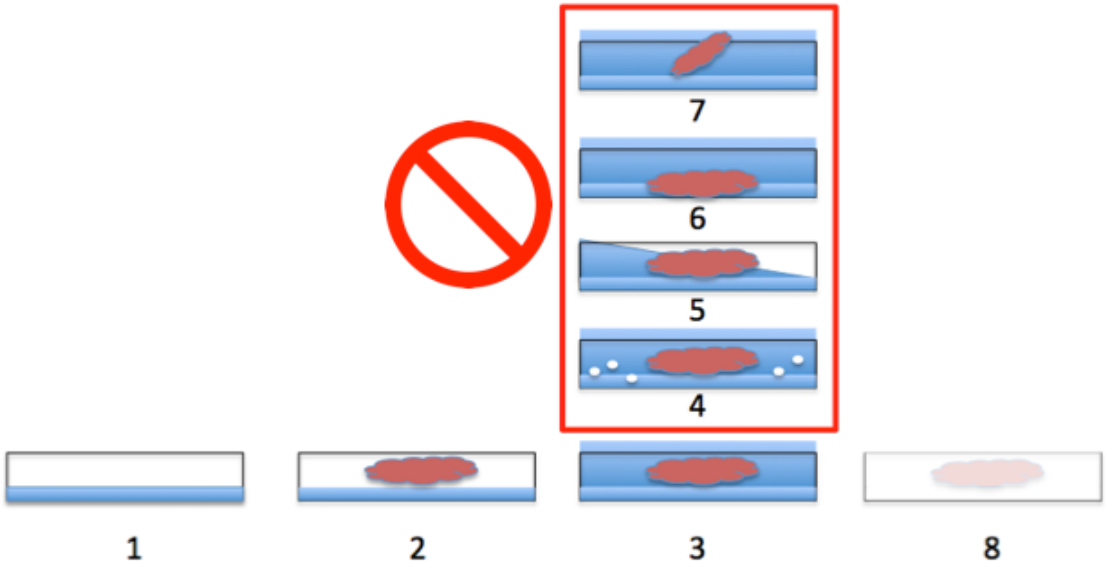
7.2.3.2. In the event that it is not feasible to provide adjacent formalin-fixed tissue for QC of the corresponding frozen tissue, pre-screening of frozen section slides for confirmation will be required. In such circumstances, the 1.0 x 1.0 x 0.5 cm tumor tissue segments are to be frozen in OCT as follows.

7.2.3.3. Loading Tissues into CryoCooler and Freezing

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (e.g., Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue embedding and freezing. Label each cryomold with an appropriate ID and "T" to indicate tumor tissue or "N" to indicate normal tissue. Each cryomold shall have a unique ID.
- Add OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) to the cryomold so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.
- Weigh and record the weight of each cryomold containing the thin layer of OCT.
- Divide the tumor tissue which is designated for research purposes after processing for clinical management into segments that are no larger than 1 cm x 1 cm x 0.5 cm. Depending upon the size of the tumor, 2-4 such tumor tissue segments may be procured, embedded in OCT, and frozen.
- Gently place each tissue segment in the well of a cryomold. The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6). Place the tissue flat in the cryomold, with its largest two dimensions sitting parallel to the bottom of

the mold. Avoid placing the tissue in the mold in any other orientation (Fig. 7). Each of the individual tissue segments should be placed in a separate cryomold.

- Quickly weigh each cryomold with OCT and tissue, before adding any additional OCT. Subtract the initial weight of the mold plus OCT (Fig. 1) from the weight of the mold plus OCT plus tissue (Fig. 2) to calculate the weight of the tissue segment. Record the weights either on paper CRFs or directly in the CDR.
- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the CryoCooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue (Fig 5). After 3-5 minutes, the OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).
- While the tumor specimens are freezing, label aluminum foil and a tissue bag with the corresponding IDs and pre-chill on dry ice.
- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.
- Record time when segments are frozen. No more than 30 minutes should have elapsed from the time of devascularization to freezing.
- Store specimens in vapor phase liquid nitrogen until shipping.



Figures 1-8. Steps for embedding and freezing fresh tissue in OCT compound. See protocol. Figures 1, 2, 3, and 8 represent the proper steps for embedding. Figures 4-7 denote improper occurrences during embedding- (4) air bubbles in OCT compound; (5) OCT not evenly covering tissue; (6) tissue sitting against floor of cryomold; (7) tissue not oriented flat in the mold.

7.2.3.4. After all tumor tissue has been frozen, snap freeze the normal tissue segments, keeping in mind that ALL tumor tissue must be frozen within 30 min and normal tissue frozen within 35 min of devascularization of the gross specimen.

- Normal adjacent tissue samples shall be frozen in the manner the tumor tissue was frozen for the case. If tumor tissue was frozen in a cryosette without the use of OCT, similarly freeze the normal tissue segment within a pre-chilled, labeled cryosette by immersion in vapor phase liquid nitrogen for 3 to 5 minutes. If OCT was utilized for the freezing of the tumor specimen, then the OCT protocol should be followed as noted in section 7.2.3.3.
- Store specimens in vapor phase liquid nitrogen until shipment.

7.2.3.5. After all tissue (tumor and normal) has been frozen, use institutional procedures to formalin fix the adjacent tissue as noted above and process to FFPE blocks to facilitate QC slide review.

7.3. Collection of Specimens for cases of AML

Both frozen tumor cells and a source germline DNA (skin punch, or 20 ug DNA from buccal swab or buccal mouthwash sample) must be available for every case.

7.3.1. **Primary tumor samples:**

- De novo non-sorted specimen (primary, untreated malignancy).
- Tumor source must be bone marrow or peripheral blood; bone marrow is preferred
- Ficoll-preparation strongly preferred (sorting or enriching cases is exclusionary)
- Post-Ficoll Smears or cytospins created of the actual sample used for banking of cells should be prepared
- 10^7 total cells required; 20^7 cells preferred
- Snap-frozen in vapor phase of liquid nitrogen

7.3.2. **Normal tissue:**

- A frozen sample of normal tissue or a buccal swab must be available for each case for the purpose of obtaining germline DNA. In order of preference, the following are suitable: Skin punch, buccal swab DNA, or buccal mouthwash DNA.
- Skin punch samples must be sufficient to yield at least 20 micrograms of DNA (6mm or greater).
- If buccal swabs or buccal mouthwash are used as source of normal tissue, DNA must be extracted prior to submission and 20 micrograms should be prepared.

8.0 Case Submission and Qualification

8.1. Biospecimen Quality Control

8.1.1. **Surgical tissue Review**

Prior to shipment of specimens to the BCR, the Tissue Source Sites (TSS) will perform a QC review of tumor biospecimens (either by review of slides from the FFPE QC blocks or review of frozen sections from the tumor blocks themselves). For each tumor segment, H&E-stained slides are to be prepared and reviewed by the TSS pathologist to confirm accuracy of tissue diagnosis and tumor composition. The PRC will further review the quality of all H&E-stained slides and compare results to that of the TSS pathologist and uploaded de-identified surgical pathology report. Any discrepancies will be arbitrated as outlined in applicable project SOPs. An assessment should be made as to whether the case meets the project requirements of:

- Primary tumor diagnosis of sarcoma, pancreatic ductal adenocarcinoma, head and neck squamous cell carcinoma, cutaneous melanoma, non-small cell lung carcinoma (lung adenocarcinoma or squamous cell carcinoma), uterine corpus endometrial carcinoma, clear cell renal cell carcinoma, or glioblastoma multiforme.
- Specimen composition:

  The cellular composition of tumor samples must be known or evaluable. By default for any cancer, the following tissue cellular composition cutoff values should be used unless otherwise specified. Note, however, that cancer-specific values are subject to change at the discretion of the NCI, as dictated by CPTAC goals and technological requirements.

  - Each tumor sample will be composed predominantly of histologically viable appearing tumor cells, as indicated from the top and bottom sections.
  - ≥50% total cellularity - of the total sample area of the histological slide, at least 50% must be comprised of viable cells (tumor or otherwise), not extracellular matrix.
  - ≥80% tumor cellularity - Of viable cell nuclei present, ≥ 80% should be tumor nuclei, and ≤ 20% of viable cells present may be normal, stromal, inflammatory or immune cells
  - ≤20% necrosis (by surface area of the entire slide)

  The CPTAC PRC will provide a further review of all generated slides and may perform additional quality checks.
- The pathology assessment is to be recorded on the Local Pathology Report Form either on paper CRFs or within the CDR. Either the reviewed H&E stained slides or whole slide digital images of the slides are to be submitted to the BCR at the time of case shipment for case qualification.

8.1.2. AML Samples

Tumor biospecimens are to be prescreened by the TSS to meet CPTAC specifications. Prescreening should be performed on a cytospin taken post-Ficoll of the sample that would be sent to the BCR for processing.

- Of cells assessed, on average, ≥ 20% must be blasts, with more homogenous samples preferred.

8.2. Tissue procurement and shipping are dependent upon fulfilling the following requirements.

- Signed patient consent (maintained at the tissue source site, applicable annotation recorded within the CDR).
- Case managed per protocol.
  - Blood collected per protocol.
  - Excision and division of the tumor and normal tissue to be performed as per SOPs.
    - Normal tissue per protocol, with the exceptions noted in Appendix B.
    - Primary tumor collected per protocol, with exceptions as noted.
  - Pathology evaluation of each tumor aliquot at the TSS, confirming diagnosis and meeting required tissue quality parameters for cellularity and necrosis.
- Adherence to BCR shipping instructions (the BCR will provide the shipping cryoport and cover the cost of shipping).
- Shipping Manifest completed and accompanying tissue shipment.
- Diagnostic pathology report uploaded to the CPTAC CDR.
- Stained slides or slide images submitted to the BCR.
- CPTAC Submission Case Report Forms (contain details regarding procurement such as ischemic time along with minimal patient information) are to be completed and electronically submitted to the CDR within 4 working days after tissue procurement and prior to shipment. Secure access to the electronic clinical data management system with the form to be provided by the CPTAC CDR.

8.3. Tumors will be qualified as acceptable for the study by:

- Matching a study tumor type identified in Table 1.
- Obtaining the minimum mass of tumor tissue required and freezing the tissues within 30 minutes from devascularization.
- Obtaining normal adjacent tissue, as applicable (see Appendix B).
- Obtaining case-matched blood, as applicable.
- TSS and PRC Pathology examination of tumor segments confirming diagnosis and meeting tissue quality parameters as specified above.

Note: Tumor tissues must be frozen within 30 minutes from devascularization in order to be considered for inclusion in the CPTAC program.

8.4. Tissue Analysis and Endpoint Measurements

All biospecimens collected will be shipped to and stored at the CPTAC BCR. The BCR will coordinate shipment of all biospecimens for analysis. In order to reduce batch variability, all samples from an individual case shall be processed together with as many cases as possible included in the same batch at the laboratory. All samples will be de-identified so that the laboratory is blinded to which samples received which treatment.

9.0 Data Collection and Management

Data for the CPTAC study will include a Limited Data Set (LDS), as defined by the Health Insurance Portability and Accountability Act (Privacy Rule). Subjects are required to give authorization for access to their medical records for abstraction of the requested data and for follow up survival data at a 12-month interval. Data elements such as clinical dates (mm/yy) will be obtained for the study. Data will be entered either onto paper Case Report Forms (CRFs) or directly onto electronic CRFs (eCRFs). Data Managers will review all data for entry errors, inconsistencies, and omissions. All deficiencies will be documented and reported to the TSS for correction and/or documentation using Data Correction Forms (DCFs).

Data collection will include:

- Biospecimen collection, handling, and processing data will be collected real-time and entered into the CDR system.
- Clinical data will be abstracted from source documents (medical records) and real-time interview with donors at the time of consent and will include donor demographics, medical history information, surgical procedure information, and the final pathology report.
- All data will be entered onto eCRFs in accordance with the Statement of Work.

9.1. Source Documents

Data will be obtained from source documents, which are considered the original records of clinical assessments or other findings requested during the conduct of this protocol. Source data are contained in source documents. Examples of source documents include: hospital or clinical records/charts, laboratory findings, participant's diaries or self-recording reports, pharmacy dispensing records, diagnostic testing files (ECHO, ECG, MRI, biopsy, etc.).

9.2. Case Report Forms

Data will be recorded on CRFs, which are the primary data collection instruments for the protocol. Data requested on the CRF will include required and supplemental information although the TSS is expected to obtain as much data as possible. Data will be entered either onto paper CRFs or directly onto electronic CRFs (eCRFs) and maintained for data record retention throughout the conduct of the study. All data recorded for this study (paper or electronic) will be reviewed and quality controlled by the site prior to the case completion.

All associated hard copy records, including completed paper CRFs (when used), will be maintained at each study site in a Project Master File (PMF). Each PMF will be available for audit by LBR and the study sponsor.

9.3. CPTAC Comprehensive Data Resource (CDR)

All requested data elements will be entered into the CPTAC CDR, an electronic data capture system, which adheres to the requirements set forth by the FDA guidance on Electronic Data Capture (EDC [Guidance for Industry, Electronic Source Data in Clinical Investigations, FDA, September 2013]). EDC will be monitored by the CPTAC CDR. The data system includes password verification, role based privileges, and follows the general workflow. Additional quality checks, such as validation and range checks to identify data that appear inconsistent, incomplete, or inaccurate will be completed both by the TSS and again by the CPTAC Data Managers. Queries will be forwarded to the TSS on a continuous basis to ensure data completeness and accuracy.

Submission Data collection and entry into the CDR should be completed within 21 calendar days from the date of surgery. All data will be stored on a central server and reviewed to ensure compliance with protocol and ethical related requirements.

Data that is entered into the CDR is in a LDS format and contains HIPAA identifiers. Only approved CPTAC project, Data Managers, Quality and CDR staff personnel have access to the full LDS data set. In order to export the data to external users, the CDR completely de-identifies the data and exports it in a coded format.

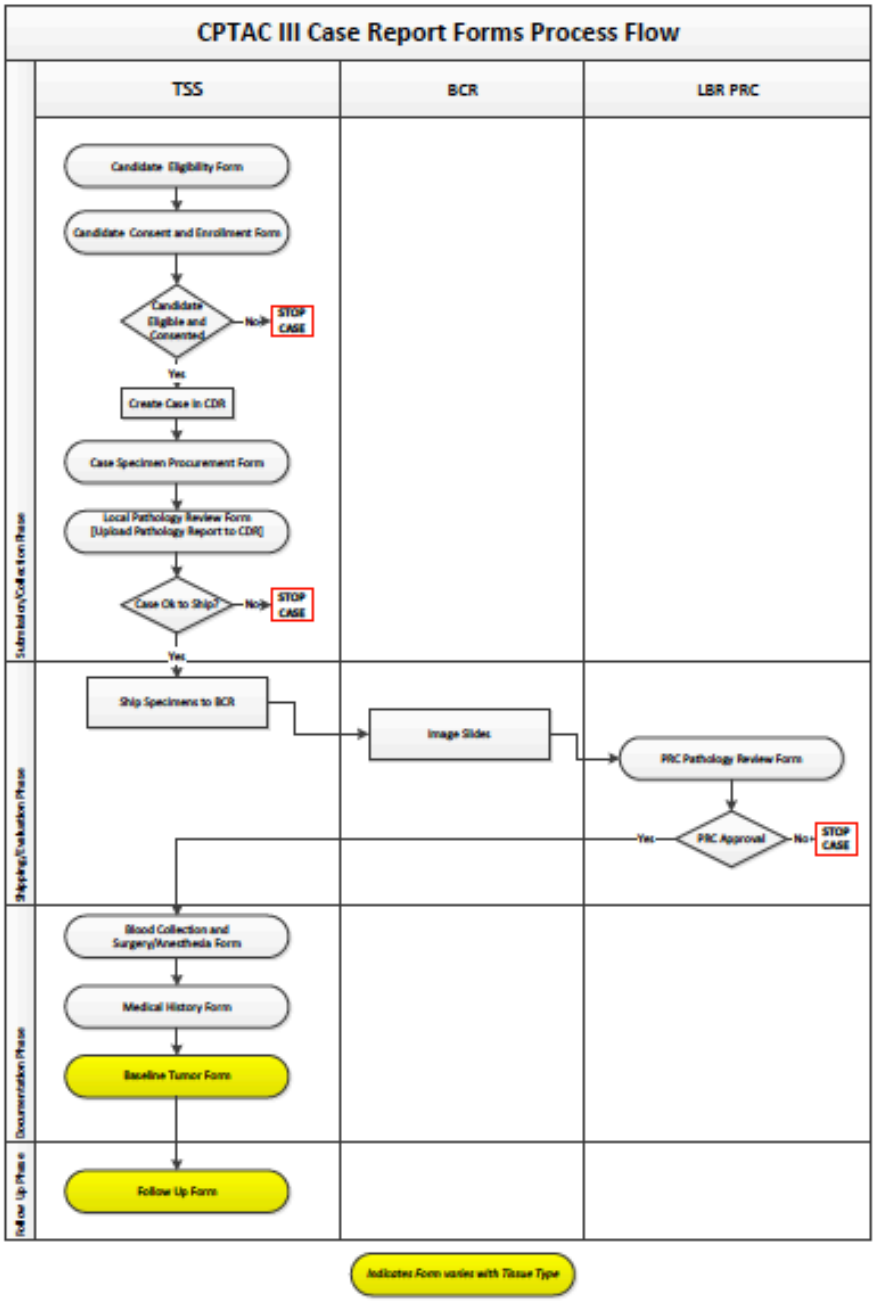


Diagram 2: Data Management Process Flow

9.4. Assignment of a Subject Code and Data De-Identification

All study subjects, associated biospecimens, biospecimen derivatives and associated subject clinical and biospecimen data will be assigned a randomly generated identification code (Candidate ID). Each collection site will maintain an enrollment log linking study subject identity to the Candidate ID assigned by CPTAC BCR. Once a subject has consented to participate in the study the Candidate ID is linked to a study subject code (CPTAC case ID) that is also randomly generated to protect the identity of the patient. CPTAC CDR and CPTAC BCR will not have access to the link between Candidate ID and patient identity. Subject CPTAC case IDs and biospecimen identification numbers will be automatically assigned via supporting database and software or provided biospecimen collections kits (pre-printed labels). All communications with the collection site will occur via the CPTAC ID. CPTAC and the CPTAC staff will never attempt to contact donor or donor families directly.

9.5. Security and Backups

All computers used for entry of data for the CPTAC study are to be password protected and only accessible to approved project study personnel. All data entered into the EDC are copied to a secure back-up server at off site several times per day and can be retrieved as needed.

10.0 Ethical, Legal and Regulatory Affair Considerations

10.1. Ethical Committee Approval

IRB approval must be in place prior to initiation of the project. All IRB submissions and subsequent amendments must be reviewed and approved by Leidos Ethical Legal Regulatory (ELR) prior to submission to the IRB. All subject screening, enrollment, biospecimen collection, and data collection will be carried out under an IRB approved study protocol. Study conduct must be in accordance with all local, state, federal laws regulations and policies for the protection of human subjects in research. The project must abide by its institution's policies and procedures, in addition to CPTAC's in order to fulfill the goals of the project. The site is required to maintain current IRB approval and the associated documents for study conduct throughout the life of the project. All documentation verifying IRB approval must be submitted to Leidos Biomed prior to initiation of the project. Amendments or modifications to the protocol, informed consent document or associated forms must first be approved by the IRB prior to implementation. Continuing reviews, amendments, and any other submissions to the IRB related to this protocol must be reported in a timely manner.

10.2. Informed Consent

All study subjects will provide prospective informed consent for biospecimen and data collection. Study participants may withdraw their participation at any point during the study. Participants have the right to discontinue participation in (withdraw) the research protocol at any time for any reason (45 CFR 46.116(a)(8)), without any prejudice to their medical care. If specimens have been sent for analysis to the GCC and/or PCC, the case will not be available for withdrawal.

Participants may be withdrawn from the protocol for any of the following reasons:

- Participant's request
- Participant's unwillingness or inability to comply with the protocol requirements
- Discretion of the PI for any reason

10.3. Replacement of Withdrawals

Participant withdrawals will be replaced if additional specimens need to be collected to meet the statistical requirement for the study or if additional biospecimens are required for analysis.

10.4. Material Transfer and Data Use Agreements

All parties that provide or receive biospecimens or use and disclose subject data related to the subject or biospecimens will be covered under a Material transfer and/or Data Use agreement, as applicable. The agreement will identify parties whose responsibilities are to protect the biospecimens and data as outlined in the terms and conditions of the agreement and in accordance with the IRB approved study protocol and the informed consent documents.

10.5. Study Contacts, Delegation of Authority, Training and Auditing

Each study site will provide an organization chart for local staff involved in the study and delegation of authority documentation showing the responsibilities delegated to local staff by the principal investigator.

Each study site should be prepared to undergo one or more study training or initiation visits by designated Leidos Biomed CPTAC staff prior to the start of biospecimen and data collection for the study, and again if substantial changes are introduced. The objective of training will be to review biospecimen collection and handing requirements and proper database usage. Copies of study staff trainings (human subject protection, IATA (shipping of hazardous and biological materials) and HIPAA) will also be required prior to project start, as applicable.

Each study site will be subject to audit(s) for compliance with NCI Best Practices for Biospecimen Resources, the CPTAC protocol and procedures and site-specific QMS program/procedures. During an on-site audit visit CPTAC personnel will require access to the entire study dataset, raw data and relevant documentation.

11.0 Protocol Management

11.1. Protocol Deviations

This study will be conducted under Quality approved SOPs that are standardized across all study sites. Sites are required to follow SOPs as written, unless otherwise instructed under a planned deviation. Any change from established SOPs will be reported as a deviation event within 3 business days of the discovery of the event. Evaluation of the deviation event will take place in order to determine root cause and evaluate impact. A final summary of the deviation event and its associated evaluation will be provided within 60 days of the event. Send deviation reporting as outlined herein by email to: NCI Leidos Biomed Quality ncileidosbiomedquality@mail.nih.gov.

11.2. Protocol changes

Future protocol enhancements or changes in study objectives can be accomplished by revision or amendment of this protocol. The study site staff must first seek Leidos Biomed approval from their Technical Project Manager prior to initiation of any changes to the protocol or approved SOPs, except in exceptional circumstance to protect study participants and minimize risks and harm. Amendments and revisions to this protocol must also first be approved by the TSS IRB prior to implementation unless in exceptional cases as stated above.

Appendix A. Reference Documents and Addenda

CPTAC Standard Operating Procedures (SOPs) – To be developed by Leidos Biomed in conjunction with the TSS

CPTAC SOP: CPTAC Candidate Screening, Consent and Enrollment

CPTAC SOP: CPTAC Blood Collection

CPTAC SOP: CPTAC Specimen Collection and Processing

CPTAC SOP: CPTAC Local Pathology Review and Sample QC

CPTAC SOP: CPTAC CDR User's Guide

CPTAC SOP: CPTAC Kit Receipt, Supplies, and Shipping Procedure

CPTAC SOP: Withdrawal of Biospecimens and Data from CPTAC Procedure

Appendix B- Table Listing Normal Tissue Exceptions

For some tumor types, normal adjacent tissue may not be available. Tumor types with this exception are listed here.

TCGA code	CPTAC code	Cancer	Alternatives to Normal
SARC	SAR	Sarcomas	
PAAD	PDA	Pancreatic Ductal Adenocarcinoma	
HNSC	HNSCC	Head and Neck Squamous Cell Carcinoma	
SKCM	CM	Cutaneous Melanoma	
LUSC	LSCC	Lung Squamous Cell Carcinoma	
LUAD	LUAD	Lung Adenocarcinoma	
UCEC	UCEC	Uterine Corpus Endometrial Carcinoma	Normal adjacent tissue is normal uterine endometrium
KIRC	CCRCC	Clear Cell Renal Cell Carcinoma	
LAML	AML	Acute Myeloid Leukemia	A bone marrow aspirate collected during remission may be subsequently submitted as a normal specimen
GBM	GBM	Glioblastoma Multiforme	Normal adjacent tissue is not required. ≤50% necrosis is acceptable.